

PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Semen quality of fertile Japanese men:a cross-sectional population-based study of 792 men
AUTHORS	Iwamoto, Teruaki; Nozawa, Shiari; Yoshiike, Miki; Namiki, Mikio; Koh, Eitetsu; Kanaya, Jiro; Okuyama, Akihiko; Matsumiya, Kiyomi; Tsujimura, Akira; Komatsu, Kiyoshi; Tsukamoto, Taiji; Itoh, Naoki; Naka-Mieno, Makiko; Vierula, Matti; Toppari, Jorma; Skakkebaek, Niels; Jørgensen, Niels

VERSION 1 - REVIEW

REVIEWER	Michael Eisenberg, MD Director Male Reproductive Medicine and Surgery Assistant Professor Department of Urology Stanford University School of Medicine USA
REVIEW RETURNED	26-Oct-2012

GENERAL COMMENTS	<p>The authors have systematically evaluated and presented semen values for fertile men in Japan. I have only minor suggestions.</p> <p>1. Were hormones evaluated only in the morning or throughout the day? Certainly there can be variations in levels in this aged population.</p> <p>2. Did men with varicoceles have lower serum testosterone levels? While not the focus of the manuscript, it would be useful data for those exploring varicoceles and would fit well with the other data presented.</p>
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REVIEWER	Ajay Nangia MBBS University of Kansas Medical Center
REVIEW RETURNED	04-Nov-2012

THE STUDY	<p>Paper needed to discuss the paper by Cooper et al in Human REproduction Dec 2009 - on the new WHO criteria.</p> <p>Inclusion/exclusion criteria. The authors should have only included men that strictly had no factors and defined pregnancies in less than or equal to 1 year. This would have made it comparable to current standards and the WHO criteria definition</p>
RESULTS & CONCLUSIONS	They should have looked at greater or less than 4% morphology not 5%

VERSION 1 – AUTHOR RESPONSE

Response to the comments from
Dr. Michael Eisenberg

We thank you for your thoughtful and constructive comments and suggestions. Here we address each of them point by point. We have revised the manuscript accordingly.

COMENT: Were hormones evaluated only in the morning or throughout the day? Certainly there can be variations in levels in this aged population.

RESPONSE: The referee is right that hormone values show diurnal variation with higher testosterone levels in the morning than in the evening. In this study, however, blood samples for hormone analysis had to be collected throughout the daytime. We could not limit the sampling time in the morning, because most of the participants were at full-time jobs that did not allow morning visits in the research laboratory. Therefore, we have added “throughout the day time” to the METHODS (p.7, line 22) and the following text to the DISCUSSION (p. 11, lines 9-13).

“Levels of reproductive hormones varied between centres, and the reasons for this are not currently known. We could not standardize the sampling time to morning, because most of the study subjects were employed in full-time jobs, and therefore blood samples were taken throughout the day. We tried to account for this by adjusting for hour of blood sampling in the statistical analyses, similar to how it has been done in other studies.^{12 21 22 24 32 33}”

Relating to the above, the following two new references have been added to the REFERENCES and have sited in the text as reference numbers 32 and 33 (p.11, line 13).

32. Paasch U, Salzbrunn A, Glander HJ, Plambeck K, Salzbrunn H, Grunewald S, Stucke J, Vierula M, Skakkebaek NE, Jørgensen N. Semen quality in sub-fertile range for a significant proportion of young men from the general German population: a co-ordinated, controlled study of 791 men from Hamburg and Leipzig. *Int J Androl.* 2008;31:93-102.

33. Fernandez MF, Duran I, Olea N, Avivar C, Vierula M, Toppari J, Skakkebaek NE, Jørgensen N. Semen quality and reproductive hormone levels in men from Southern Spain. *Int J Androl.* 2012;35:1-10.

We have also added the following information to the Statistical analysis section in the METHODS (p.9, lines 1-5) how this was accounted for in the analyses. Previously, this was only available in a footnote of Table 2.

“Reproductive hormone levels, i.e. FSH, inhibin-B, LH, testosterone, SHBG, and calculated free-testosterone, were also log-transformed, and the between group differences were tested by multiple linear regression adjusted for age, BMI, season, and blood-drawn time. BMI was entered as linear and quadratic form, and when reporting the adjusted values, ‘32-year-old man with a BMI of 23 and blood sampling at 10:00 am in winter season’ model was represented”

COMENT: Did men with varicoceles have lower serum testosterone levels? While not the focus of the manuscript, it would be useful data for those exploring varicoceles and would fit well with the other data presented.

RESPONSE: No significant differences were observed in serum testosterone levels between the two

populations of the fertile men with and without varicocele. Therefore, we have changed the following text in the RESULTS from “Neither FSH nor inhibin-B differed from others” to “Serum levels of FSH, Inhibin-B and testosterone did not differ from others” (p.10, lines 1-2)

Response to the comments from
Dr. Ajay Nangia

We thank you for your thoughtful and constructive comments and suggestions. Here we address each of them point by point. We have revised the manuscript accordingly.

COMENT: Paper needed to discuss the paper by Cooper et al in Human REproduction Dec 2009 - on the new WHO criteria.

RESPONSE: The present study was conducted in an international collaborative project as a comparative study with the previously published European study (Jorgensen et al., 2001: ref. 21) and a Japanese study (Iwamoto et al., 2006: ref. 24). We therefore used those criteria to allow comparison with the previous studies. We have now added the following text to the DISCUSSION (p.10, lines 14-21) about the new WHO (2010) criteria and the paper by Cooper et al., 2010.

“WHO published the latest guidelines and reference values of semen analysis 2010.²⁸ These are based on several prospective cross-sectional studies of fertile men whose partner conceived within 12 months after stopping contraception. However, the background publications did not all request conception within 12 months, eg. our own,²¹ but data on time to pregnancy was available.²⁹ The new reference values define the 5th percentile of the fertile men, and those are now clearly lower than previous values.²⁵ Interestingly the present Japanese semen values are close to the WHO reference values, and the 5th percentiles for sperm concentration and total sperm count are 18 million/ml (WHO reference 15 million/ml) and 38 million (WHO reference 39 million), respectively”

Relating to the above correction, the following two new references have been added to the REFERENCES and have cited in the text as reference numbers 28 and 29 (p. 10, lines 17 and 18).

28. World Health Organization. WHO Laboratory Manual for the Examination and Processing of Human Semen, 5th edn. 2010 WHO Press, Geneva.

29. Cooper TG, Noonan E, von Eckardstein S, Auger J, Baker HW, Behre HM, Haugen TB, Kruger T, Wang C, Mbizvo MT, Vogelsong KM. World Health Organization reference values for human semen characteristics. Hum Reprod Update. 2010;6:231-45.

COMENT: Inclusion/exclusion criteria. The authors should have only included men that strictly had no factors and defined pregnancies in less than or equal to 1 year. This would have made it comparable to current standards and the WHO criteria definition

RESPONSE: We appreciate the need for standardization in inclusion criteria. Inclusion/exclusion criteria of this study were determined at the same time when the comparative European studies started in the mid 1990s, i.e. long before the current WHO guidelines were available. The men were recruited in the antenatal clinics and therefore they had recently shown to be fertile. Furthermore, 45.1% of them had already previously fathered a child. According to the time to pregnancy data that we collected, the pregnancy had been conceived in 87.4% in less than or equal to 12 months. This has now been added to the Methods section. “The factors” were clearly described in Table 1. It is always a compromise to use different inclusion/exclusion criteria, and here chose to allow a valid comparison to the previous Japanese study and to the European studies rather than to WHO criteria that were published after our study was already finished. Therefore, we have added the following

information to the METHODS.

“Characteristics of the study populations are given in Table 1” (p.6, line 9)

“According to the time to pregnancy data that was available from 523 couples except for those from Sapporo, the pregnancy had been conceived in 87.4% in less than or equal to 12 months” (p.6, lines 15-16)

COMENT: They should have looked at greater or less than 4% morphology not 5%

RESPONSE: Here we also wanted to compare the results with the previous European studies that used the very same protocol as the present study used. The criteria for the reference limits of normal semen quality have changed over the years (to lower and lower values), and here we wanted to stick to the values that were normative at the time of the study. We have now also included the number of men below the 4% level. Therefore, we have changed the text in the RESULTS (p.9, lines 19-20) from “For morphology 14.4% had less than 5% normal spermatozoa” to “For morphology 14.7% had less than 5% and 10.4% less than 4%” (The change from “14.4%” to “14.7%” is a simple correction of a typing mistake).

COMENT: Issues of inclusion/exclusion criteria do not make the data credible.

RESPONSE: Here we have to courteously disagree with the reviewer. The inclusion/exclusion criteria of this study were the same as in the previous European study that used the same protocol and was published in Human Reproduction in 2001. The results of these studies were also included in the publication of the WHO reference levels. We have clearly defined our study population and do not make any statements about possible semen quality in other than that. Therefore it is difficult for us to see why the data would not be credible in this well-defined population.

Other corrections

- We found a mistake in the adjusted values of the sperm concentrations in Table 2, which has been changed to the following revised version.

- A minor revision: We have changed the text in the Results (p.9, line 16-17)

From:

“Semen volumes, percentages of motile spermatozoa and morphologically normal spermatozoa differed between the four groups, whereas no differences in sperm concentrations or total sperm counts were found”

to:

“Semen volumes, sperm concentrations, percentages of motile spermatozoa and morphologically normal spermatozoa differed between the four groups, whereas no differences in total sperm counts were found”

- A minor revision: We have changed the word in the Results (p.12, line 9) from “possibl” to “possibly”

- In consequence of adding the four new references, the reference number 28 and later have been advanced in sequence. These changes have been presented the revised manuscript at every corresponding part.

VERSION 2 – REVIEW

REVIEWER	Michael Eisenberg Department of Urology Stanford University USA No conflicts of interest
REVIEW RETURNED	17-Dec-2012
GENERAL COMMENTS	The authors have addressed my concerns.